PERSPECTIVE

Treatment of Complex Regional Pain Syndrome (CRPS): New Perspectives in the use of Sulfonamides as Modulators of P2X Receptors

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Several disorders such as inflammation, neuropathy, or chronic pain are debilitating illnesses and remain a large unmet medical need. The drug machinery to treat these conditions often does not provide complete relief or does not provide relief in all patients. Therefore, the discovery of new chemical entities to increase the paraphernalia of medicaments is highly demanding and should be the focus of medicinal chemistry research.

Among these pathologies, Complex Regional Pain Syndrome (CRPS) is significant since a large amount of the population all over the world is affected by it, and efficacious treatment is still far away to be discovered. In this paper, an analysis of the state-of-art and perspectives for the discovery of new treatments of CRPS are highlighted.

CRPS is a broad term describing excess and prolonged pain and inflammation that follows an injury to an arm or leg. It has acute (recent, short-term) and chronic (lasting more than six months) forms; more common in women; however, it can occur in anyone at any age, with a peak around age 40. It is rare in the elderly, who have less inflammation after injury, and in young children who heal quickly and completely. Thus, the outcome of CRPS is highly variable. For the state of the art on this pathology, the interesting review published in 2015 by Stephen Bruehl in the British Medical Journal, where the point of view of patients is also reported, can be considered [1].

Generally, patients experience continuing pain that is disproportionate to any inciting event; and several symptoms are common which include hyperesthesia or allodynia, edema and/or sweating changes, weakness, tremor, dystonia, and/or trophic changes, decreased range of motion and/or motor dysfunction. Diagnosis is often difficult although the International Association for the Study of Pain ("IASP") has established diagnostic criteria for CRPS, known as the Budapest Criteria, named after the conference, where the same was established [2-3].

Of course, also therapeutic approach is not well established yet, and treatments are limited to symptom relief. A list of the most used drugs is presented in Table **1**, together with pharmacological and common side effects in the case of CRPS, when available. Several of these medicines resulted in effective CRPS treatment, particularly when given early in the disease. However, no drug has been approved so far by the U.S. Food and Drug Administration (FDA) to be marketed specifically for the syndrome, and no single drug or combination is guaranteed to be effective in every patient. All the treatments are palliative rather than curative, and usually, monotherapy is best to minimize adverse effects, and patient noncompliance. If additional pain control is needed, opioid analgesics can be used to facilitate, for example, the resumption of more normal daily activities.

However, since neurotransmitters may induce abnormal connections and signals between sympathetic and sensory nerve cells in chronic pain conditions such as CRPS, it is of great importance to understand how changes in nerve connections following peripheral nerve injury may influence the onset of pain.

A complex regional pain syndrome of an extremity, that has previously resolved, can recur after repeat surgery at the same anatomic site, therefore this pathology is often described as a disease of the autonomic nervous system [15].

As an initial step of the activation of immune mechanisms, elements of the innate immune system rapidly react to the accumulation of DAMPs (damage-associated molecular patterns).

Peripheral nerve injuries are accompanied by inflammatory reactions, the over-activation of which may hinder recovery. Among pro-inflammatory pathways, inflammasomes are one of the most potent, leading to the release of active IL-1β. Thus, preventing the development of neuronal inflammation by neutralizing IL-1β, blocking NLRP3 inflammasome (detrimental to the recovery process), or inhibiting the P2X4 receptor results in improved motoneuronal survival and motor axon regeneration following peripheral nerve co-aptation [16].

Among the wide range of DAMPs, extracellular adenosine-triphosphate (ATP) is a multi-target danger signal, which increases neuronal susceptibility to damage, and

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Table 1.   **Drugs used to treat CRPS.**

Type of Drug	Suggested Treatment	Side Effects[#]	Dose
Acetaminophen	Reduce pain associated with inflammation and bone and joint involvement	Inadvertently overdose due to ubiquitous inclusion in combination medications with opioids [4]	4 g/day
Bisphosphonates (high dose alendronate or intravenous pamidronate)*	Reduce bone changes	Low level of toxicity, osteonecrosis is a rare complication [5]	2.5 mg/day risedronate; 35 mg/day alendronate
Botulinum toxin injections	Relaxing contracted muscles and restoring normal hand or foot positions	Useful for focal dystonias limited to small areas, too invasive and expensive for widespread regional dystonias	-
Corticosteroids (prednisolone and methylprednisolone)	Treat inflammation/swelling and edema	Longer courses are unproven [6], but there are numerous, serious contraindications to chronic steroid use	Initial dose at 30 mg.
N-methyl-D-aspartate (NMDA) receptor antagonists (dextromethorphan and ketamine)	Controversial treatments	Long-term toxicity (unresolved issues) [7-8] cognitive impairment (especially with "rescue dosing"), opioid-induced hyperalgesia [9-10]	400 mg/day Dextromethorphan; 3-5 mg/kg/hour Ketamine
Nortriptyline, gabapentin, pregabalin, duloxetine, and amitriptyline**	Neuropathic pain conditions	Respiratory depression when administered alone and/or in combination with opioids [11]	50-100 mg/day nortriptyline; 900-2400 mg gabapentin; 150-600 mg/day pregabalin; 60 mg duloxetine; 75 mg/day amitriptyline
NSAIDs (Non-steroidal anti-inflammatory drugs): aspirin, ibuprofen, and naproxen	Moderate pain and inflammation, used for both prophylaxis and rescue	-	500 mg aspirin; 1200 mg/day Ibuprofen; 250-500 mg/ twice daily naproxen
Opioids (oxycodone, morphine, hydrocodone, and fentanyl)	Most severe pain	Common opioid side effects, especially at high doses (nausea, vomiting, constipation, cognitive impairment, somnolence) care must be taken to avoid drug abuse	90 mg morphine; 12 mcg/h fentanyl
Topical local anesthetic (used as sprays, or creams): lidocaine fentanyl	Reduce allodynia, and skin coverage by patches can provide additional protection	Frequent nausea	700 mg/day lidocaine

Note: [#]Mostly reviewed in references [4, 12].
*Zoledronic acid and neridronate were more recently (2013) introduced in therapy upon approval by EMA [13] and FDA [14], respectively.
**Old drug effective (dosage of 75mg a day) but with more side effects (such as constipation, headache, or dizziness) than nortriptyline, a very similar chemical analog.

induces inflammatory changes mostly through the P2X purinergic receptor subfamily [17]. Therefore, to discover new possible treatments, medicinal chemistry researchers need to continue to investigate how inflammation is related to the release of ATP which is involved with energy production within cells and can also act as a neurotransmitter.

P2X receptors are mainly involved in inflammatory disorders like pain sensation and neuroinflammation, making them a new drug target for the treatment of neurodegenerative disorders or chronic pain. The receptors belong to a family of ionotropic ATP-gated receptors and are trimeric ion channels selective to cations. Recent progress in the molecular biophysics of these channels enables a better understanding of their function [18].

The distribution and localization of subtypes of P2X receptors depend on their capability to pass through secretory and endocytic pathways: thus they are localized in different subcellular compartments (Fig. **1**) [19]. Some are retained within the endoplasmic reticulum (ER), while others are predominantly at the cell surface or within endosomes and lysosomes. The up-or down-regulation of the cellular response to ATP depends mainly on changes in the recruitment of receptors to and from the plasma membrane.

Among the P2X family of receptors, P2X4 is one of the most sensitive purinergic ones (at nanomolar ATP concentrations). In the CNS, P2X4 modulates synaptic transmission and communication between neurons and neighboring glial cells.

Table 2.   P2X4 selective antagonists.

Antagonist	Mechanism	Species Inhibition (IC$_{50}$ in μM) [12]
1 carbamazepine [32]	Negative allosteric modulator	Human (IC$_{50}$=3.44 μM), Rat (IC$_{50}$=54.6 μM), Mouse (IC$_{50}$=14.9 μM)
2 5-BDBD [33, 34]	Competitive	Human (IC$_{50}$=1.6 μM), Rat (IC$_{50}$=0.50 μM)
3 NP-1815-PX [12]	Competitive	Human (IC$_{50}$=0.3 μM), Rat (IC$_{50}$=10 μM), Mouse (IC$_{50}$=14.9 μM)
4 BX-430 [35]	Non-competitive allosteric	Human (IC$_{50}$=0.54 μM)
5 PSB-12054 [12]	Allosteric	Human (IC$_{50}$=0.19 μM), Rat (IC$_{50}$=2.13 μM), Mouse (IC$_{50}$=1.9 μM)
6 PSB-12062 [36]	Allosteric	Human (IC$_{50}$=1.38 μM), Rat (IC$_{50}$=92.8 μM), Mouse (IC$_{50}$=1.76 μM)

Adenosine-triphosphate, the well-known extracellular signaling molecule, as a neurotransmitter activates purinergic P2X receptors. After the X-ray, crystal data of P2X4 (zebrafish) were available allowing elucidation of its structure, several pieces of information have been acquired also on the gating of P2X channels, although there is still much to discover regarding the receptor behavior in the central nervous system (CNS). Interestingly, the determination of the crystal structure was obtained either in an apo, closed-channel state [20] or in an ATP-bound, presumably open-channel state [21], which allowed the delineation of the principles of ligand binding, channel opening, and allosteric modulation.

Concerning the P2X4 structure, the receptors are constituted by three subunits, each of which contains 388 amino acids. It consists of an intracellular domain (which includes N-terminus and C-terminus), two transmembrane domains (TM1 and TM2), and a large extracellular loop domain [22]. The intracellular N-terminus is short and highly conserved, whereas the intracellular C-terminus is relatively long and is mainly involved in binding to other known proteins and can affect the degree of reaction with ATP binding. TM1 and TM2 domains have α and β helix structures and regulate calcium influx: three α helices of the TM2 domain form an ion channel, which mainly affects the flow of calcium ions. The extracellular loop domain contains three ATP binding sites, formed by adjacent subunits. Interestingly, the P2X4 receptor can form a heteromultimeric structure with other P2X subtypes (P2X1, P2X2, P2X6, and P2X7) [23-25].

The involvement of P2X4 in a variety of pathophysiological processes is already well recognized, as well. For example, P2X4 receptors have been reported as implicated in neuropathic [26-29] or inflammatory pain [30].

An interesting overview of what is known about P2X4 expression in the CNS, including evidence for its role in neuroinflammation and neuropathic pain, has been reported in 2017 [31].

The discovery of selective antagonists of the P2X4 receptor would allow on one hand further elucidation of their physiological roles, on the other their use as a drug might lead to a novel therapeutic approach for several diseases, including CRPS.

Several modulators of P2X4 have already been reported in the literature. A list of the most important antagonists is represented in Table **2**, together with the nature of their function, whereas their structures are shown in Fig. (**2**).
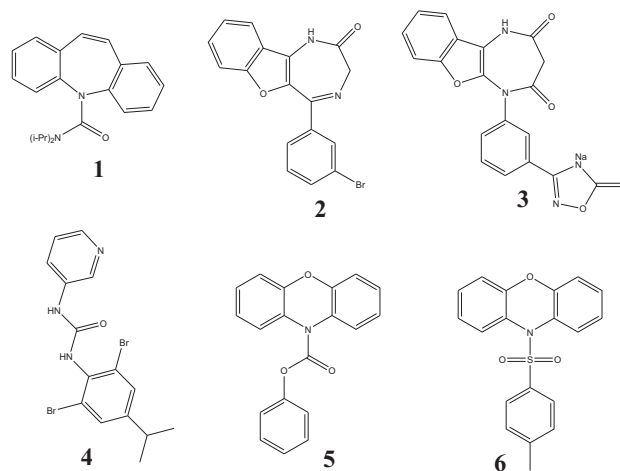
Fig. (1). Structures of P2X4 antagonists. (**1**) carbamazepine; (**2**) 5-BDBD; (**3**) NP-1815-PX; (**4**) BX-430; (**5**) PSB-12054; (**6**) PSB-12062.

From a chemical point of view, inhibitors can be classified as polycondensed azepine/diazepine derivatives (compounds **1-3**), ureido derivatives (compound **4**), and functionalized phenoxazines (compounds **5** and **6**). In our opinion, particular interest in their biological activity is the key portion of inhibitor **4**, especially the NH-CO moiety, and the sulfonamide pharmacophore in **6**.

In 2019, Werner and coworkers identified after a wide SAR (structure-activity relationship) study the potent and selective P2X4 inhibitor BAY-1797 (namely N-[4-(3-chlorophenoxy)-3-sulfamoylphenyl]-2-phenylacetamide) closely related to the known inhibitors **4** and **6** (Fig. **3**) [37].
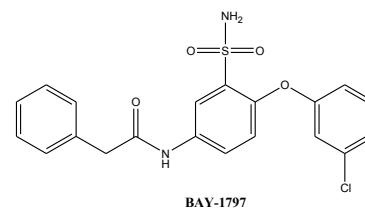
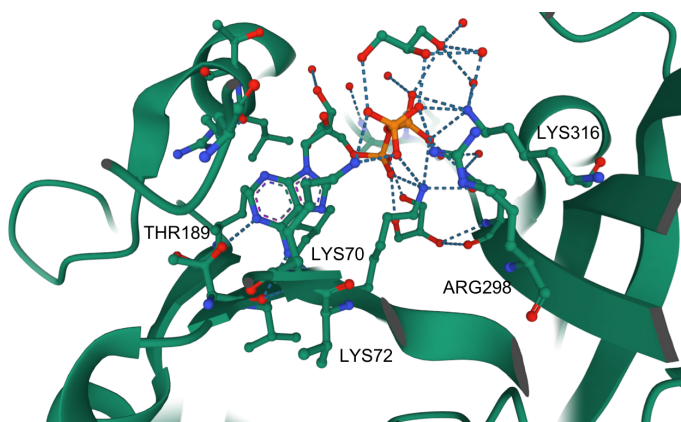
Fig. (2). Structure of P2X4 allosteric antagonist.

Fig. (3). ATP bound to P2X4 receptor (crystal structure from PDB, 4DW1). *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*
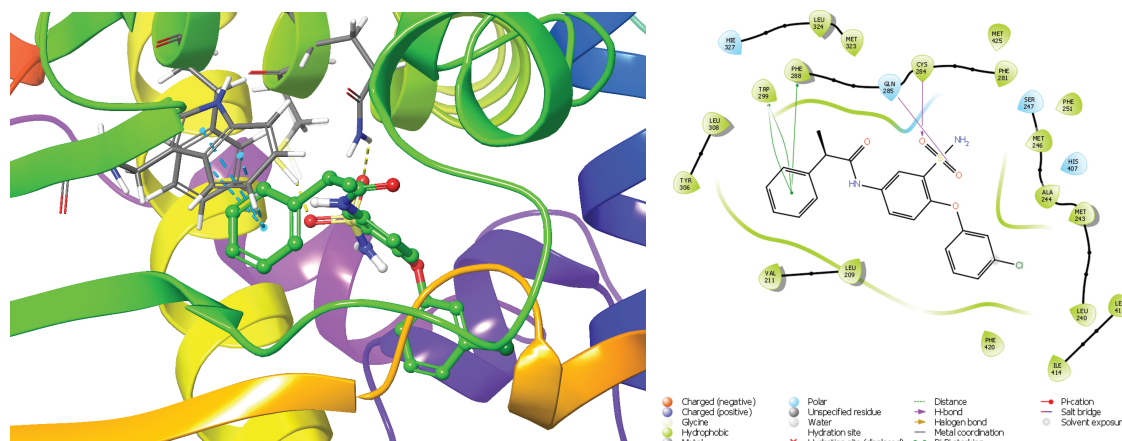

Fig. (4). 3D-Structures of ligand BAY-1797 and P2X4 receptor (PDB ID: 6HTY). In yellow dashed lines the H-bonds with Cys284 and Gln285 and the sulfonamide moiety are reported, in blue dashed lines the pi-stacking interactions with Phe288 and Trp299 (Up) are reported. 2D-ligand interaction pattern (Down). *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

When compared to other allosteric inhibitors, the recently described sulfonamide derivative BAY-1797 resulted in very selective against P2X4, being similarly potent at the human, rat, and mouse P2X4 receptors [(Human (IC_{50}=211 nM), Rat (IC_{50}=233 nM), Mouse (IC_{50}=112 nM)], but moderate in potency. Due to its polar character, it is well water-soluble and does not penetrate well into the brain, being thus only peripherally active. Therefore, this compound is suitable for *in vivo* studies in rodents, and the anti-inflammatory and antinociceptive effects of BAY-1797 were demonstrated in a mouse complete Freund's adjuvant (CFA) inflammatory pain model, behaving as a negative allosteric modulator of P2X4.

Additionally, the activity of the P2X4 receptor is also modulated by other allosteric regulators, the most common of which are:

-Metal ions, such as Zinc ions can enhance the activity of the receptor, while Copper ions have the opposite effect [38];

-pH value, considering that acid conditions can reduce/inhibit its activation, and *vice versa* [39];

-Ethanol concentration, by increasing it the activity decreases, also in a dose-dependent fashion [40].

Therefore, to better understand how the antagonists can interact with the receptor and interfere with its activity, resulting in thus useful for the treatment of CRPS eventually,

in silico experiments can open the way to fast and not expensive selection of new scaffolds. It is well known that using several protocols such as docking, induced-fit docking and molecular dynamics, the development of new entities can be undertaken; in fact, all these techniques have been successfully employed to study several types of complex ligand-receptor interactions, also by our research group [41-43].

Starting from the structure of the zebrafish P2X4 receptor, obtained by X-ray analysis, the trimeric structure and the location of the orthosteric binding site within each of the three subunits have been confirmed (PDB ID: 4DW1) [11, 20]. In this ATP-gated P2X4 ion channel in the ATP-bound, open state, ATP was found to bind in an unusual U-shaped conformation (Fig. **4**). The ligand is held by several interactions with key residues: in particular polar interactions were evidenced between the anionic γ -phosphate group of ATP with basic amino acid residues (Lys72, Arg298, and Lys316), reinforced by hydrogen bonds (H-bonds) of the N6-amino group of ATP with Lys70 and Thr189 [44].

Among the other crystal structures available from the PDB, the crystal structure of P2X4 (PDB ID: 6HTY), complexed with the inhibitor BAY-1797 (Fig. **5**), evidenced the presence of several H-bonds, which stabilize the complex, especially involving the sulfonamide moiety [37].

Table 3. **Representative examples of biologically important sulfonamides and the treatments for which they are indicated.**

Sulfonamide	Therapeutic Use	Clinical Trial*	PK	PD
Abrocitinib	Atopic dermatitis	[47]	$t_{1/2}$=4.85 h	IC$_{50}$=29.0 nM
Acetazolamide	Idiopathic intracranial hypertension	[48]	$t_{1/2}$=13,1 h	IC$_{50}$=
Amprenavir	Anti HIV	[49]	$t_{1/2}$=7-10 h	IC$_{50}$=0,41-0,012 µM
Baricitinib	Rheumatoid arthritis	[47]	$t_{1/2}$=7.28 h	IC$_{50}$=5.9 nM
Brinzolamide	Glaucoma, ocular hypertension	[50]	$t_{1/2}$=111 days	IC$_{50}$=3.19 nM
Glibenclamide	Cerebral edema	[51]	$t_{1/2}$=	EC$_{50}$=48 nM
Indisulam	Anticancer	[52]	$t_{1/2}$	IC$_{50}$=0.10-4.4 µg/mL
Pazopanib	Advanced renal cell carcinoma	[53]	$t_{1/2}$=35 h	IC$_{50}$= 7 nM
Sulfasalazine	Rheumatoid arthritis	[54]	$t_{1/2}$=3.6 h	IC$_{50}$=
Sulpiride	Antipsychotic	[55]	$t_{1/2}$=6-8 h	IC$_{50}$=10.28 nM

Note: *Main results of these studies are discussed in the reported references.
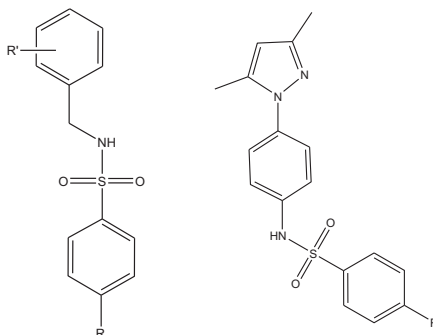
Fig. (5). Structures of sulfonamides proposed as modulators of P2X4 receptor.

Another interesting piece of evidence is from Seguela and colleagues that taking advantage of high-resolution crystallographic data available on zebrafish P2X4, used molecular dynamics simulation to model the docking of BX430 on an allosteric binding site around Ile315 [45]. Moreover, as evidenced in the complex (PDB ID: 4DW0), Tyr303 and Asp91 residues also contribute to the binding of the inhibitor, which occupied the allosteric antagonist-binding site.

The class of sulfonamide compounds is very versatile from the pharmacological utility point of view [46]. A short list of sulfonamides of importance in pharmacology is presented in Table **3**, together with some important physicochemical properties.

In this context, the availability of suitable tool compounds and drugs will help to advance basic research and target validation in the field. Since it was evidenced that for P2X4 receptor inhibitors, the ability to interact through H-bonds with key residues of the binding site is of primary importance, which is reinforced by ionic or coordination with metal ions by suitable pharmacophore moieties, we hypothesize that the sulfonamide derivatives, the structures of which are depicted in Fig. (**5**), already studied by us as potential inhibitors of human telomerase [56], could act as a modulator of P2X4 receptor in the perspective of treat CRPS.

Finally, again, the perspective of the discovery of a new treatment of CRPS, it is worthy to mention the very recent paper that appeared in literature by Christa Müller and Vigneshwaran Namasivayam where the structural requirements to develop selective inhibitors for P2X and P2Y as well are summarized [57].

LIST OF ABBREVIATIONS

CRPS   =   Complex Regional Pain Syndrome

ER   =   Endoplasmic Reticulum

FDA   =   Food and Drug Administration

IASP   =   International Association for the Study of Pain

NMDA   =   N-methyl-D-aspartate

NSAIDs   =   Non-steroidal Anti-inflammatory Drugs

CONSENT FOR PUBLICATION

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